

Antioxidant potential of olive leaf (*Olea europaea* L.) sustainable extracts evaluated *in vitro* and minced beef meat

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Abstract

In this work, by using water steam only, two antioxidant extracts were obtained from olive leaves (*Olea europaea* L.), a by-product of olive oil chain. Olive leaf extracts (OLEs) were tested as such (water extract: WE) and partially purified with ethyl acetate (ethyl acetate [EA] extract). Total phenols were 7.4 mg/mL and 3.8 mg/mL in WE and EA final solutions, respectively, evidencing a different composition by high-performance liquid chromatography analysis. Both extracts were evaluated *in vitro* in comparison to pure hydroxytyrosol (Hy). A 2,2-diphenyl-1-picrylhydrazyl (DPPH) EC₅₀ of 57.6, 76.5, and 39.7 µg/mL and a ferric reducing antioxidant power EC₅₀ of 84.8, 69.9, 41.2 µg/mL were determined for WE, EA, and Hy solution, respectively. The Rancimat induction time determined at 120°C in a lard sample with 200-ppm total phenol equivalent addition of WE, EA, and Hy was 8.92 h, 12.74 h, and 7.27 h, respectively (vs. 2.24 h for lard only). Extracts were added at the same dose (200 ppm) to minced beef patties that were put in closed containers under controlled air headspace composition (NA, natural air; HOA, modified air with 80% O₂; and 20% N₂) and stored at 4°C up to 10 days. Extracts showed significant effectiveness in contrasting decrease of O₂ level in containers as well as pH and color changes of patties. A significant increase (expressed as mg of malondialdehyde MDA/kg; thiobarbituric acid-reactive substance (TBARS) assay was conducted in control samples at an interval of 0–10 days (from 0.52 to 0.78 mg of MDA/kg in NA samples; and from 0.55 to 1.31 mg of MDA/kg in HOA samples), while minimal changes were observed in treated samples. These findings suggest a potential use of OLEs for maintaining quality and oxidative stability of beef patties during storage.

Keywords: minced beef meat; natural antioxidant; olive leaf extracts; oxidative stability; TBARS assay

Introduction

Olive (*Olea europaea* L.) virgin oil is gaining global recognition for numerous reasons, including its unique phenolic content, which may help to reduce the occurrence of cardiovascular diseases, neurodegenerative conditions, and certain types of cancers (Gorzynik-Debicka *et al.*, 2018). In addition, the by-products of olive oil

chain, such as leaves and olive oil mill wastes, are rich source of these or similar phenolic compounds (De Leonardis *et al.*, 2009; McDonald *et al.*, 2001; Munekata *et al.*, 2020).

The olive plant's phenolic compounds also increase the shelf life of food products, including meat products, by delaying lipid peroxidation (Djenane *et al.*, 2018,

Gómez *et al.*, 2020). Thus, they can serve as natural alternatives to synthetic preservatives, aligning with the growing consumer demand for healthier and more natural food options. Indeed, several studies have investigated the potential use of olive oil mill by-products or their extracts in food formulations, nutraceuticals, and dietary supplements as well as livestock diets to improve rumen function, milk production, and meat quality (Bender *et al.*, 2023; De Leonardis *et al.*, 2022a, 2022b; Tzamaloukas *et al.*, 2021). Moreover, phytochemicals found in olive leaves (OLs) are of high nutraceutical and pharmacological interest because of their antioxidant, free radical scavenging, anti-inflammatory, cardioprotective, hepatoprotective, and anti-cancer activities, as demonstrated in various biological systems (Pereira *et al.*, 2007; Romani *et al.*, 2019; Ruzzolini *et al.*, 2018). Oleuropein, a secoiridoid glucoside, is a major component of olive leaf phenolic fraction, followed by pure hydroxytyrosol (Hy, a phenylethanoid), apigenin 7-*o*-glucoside of luteolin, verbascoside, and other phenolic acids (Rahmanian *et al.*, 2015).

Beef is a nutritious and popular meat type processed in various forms, including patties, for convenience and diversity. Unfortunately, it is estimated that approximately 1.3 billion tons of meat, including beef, is wasted annually along the entire supply chain, mainly at the household level, globally because of poor preservation facilities (Hadidi *et al.*, 2022). Massive meat wastage is caused by microbial and chemical spoilage, resulting in food-borne illnesses and food insecurity. Chemical spoilage is mainly caused by lipid and protein oxidation, which are responsible for the production of undesirable compounds such as aldehydes, ketones, and free radicals that provoke rancidity, off-flavors, discoloration, nutritional loss, and health risks (Honikel, 2017; Lorenzo *et al.*, 2017).

The process of mincing influences the oxidation of beef because it increases the meat's surface area, resulting in higher exposure of lipids and proteins to O₂, heat, light, and metal ions. Consequently, mincing of meat to produce beef patties results in higher oxidation than whole-muscle meat, especially after cooking and storage (Shimizu and Iwamoto, 2022).

Lipid oxidation is a process that includes multiple mechanisms with very complex reactions and interactions between substrates and catalysts. These reactions are influenced by various intrinsic (meat composition) and extrinsic (processing and storage conditions) factors (Lorenzo *et al.*, 2017). Commonly, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG), act as hydrogen atom donors, establishing a crucial role in preventing or delaying undesirable changes. Additionally, nitrites and nitrates are frequently used as meat additives with the function

of protecting against lipid and protein oxidation (Zhang *et al.*, 2023). However, these synthetic additives may have deleterious consequences on health, including allergic reactions, liver damage, carcinogenicity, and DNA damage (Carocho *et al.*, 2018). Consequently, their use is closely monitored and subjected to strict regulations in numerous countries.

About the above questions, the utilization of natural antioxidants as substitutes for synthetic additives in meat and meat products has generated significant interest. Thus, many studies have investigated the efficacy of extracts derived from a variety of plant, vegetable, and fruit sources as well as lesser-explored resources, such as food industry by-products and waste (Allam *et al.*, 2024). In this context, several studies have addressed the discovery and development of natural and synthetic food additives from olive leaf to enhance the quality and shelf life of meat products (Aouidi *et al.*, 2017; Difonzo *et al.*, 2022; Djenane *et al.*, 2018; Munekata *et al.*, 2020; Totaro *et al.*, 2024).

In this study, olive leaf extracts (OLEs), obtained using water steam only as a solvent, were characterized and investigated as a potential antioxidant additive to improve the shelf life of beef patties. Sustainability has been defined as 'the development which meets the needs of the present, without compromising the ability of future generations to meet their own needs'. In the last decades, development of sustainable products in industry, energy, transport, agriculture, and tourism has become a global key issue for the society, economy and the environment. The studied OLEs may be considered sustainable for various reasons, such as recycling of agricultural waste, use of a clean solvent, and production of natural food additives. The antioxidant potential of extracts was evaluated *in vitro* by using different laboratory tests (2,2-diphenyl-1-picrylhydrazyl [DPPH] and ferric reducing antioxidant power [FRAP] assays and the Rancimat test). Moreover, the extracts were tested directly on beef patties stored at 4°C for 10 days and packaged in a natural atmosphere or modified atmosphere (80% O₂ and 20% N₂ atmosphere) to stress oxidative conditions; in this case, changes in pH, color, and thiobarbituric acid-reactive substance (TBARS) measures were monitored at 0-, 5-, and 10-day intervals.

Materials and Methods

Chemicals

All chemicals, reagents, and solvents were of analytical or high-performance liquid chromatography (HPLC) grade. Hydroxytyrosol (Hy) and oleuropein standard compounds were purchased from Sigma Aldrich Chemicals (Milan, Italy).

Olive leaf extract preparation

Fresh OLs were randomly detached manually from the cut branches of olive trees growing in the Molise Region of Italy. Approximately 500 g of whole OLs, previously frozen for 24 h to facilitate extraction, was placed on the bottom grid inside the boiler of an essential oil extractor with a capacity of 12 L (Inherbashop, Stavella, Verona, Italy). Approximately 2 L of water was heated at 100°C for approximately 30 min to produce aqueous steam; the remaining water was spontaneously cooled to room temperature and divided into two parts. One part was dried using a rotary evaporator (rotovap) at 60°C, and the solid residue was dissolved in 50 mL of 30% ethanol–water solution (v/v); this extract was called WE (water extract). The second part of the boiler water was transferred to a separating funnel and washed thrice with ethyl acetate. The collected ethyl acetate layers were evaporated to dryness using a rotary evaporator by recovering solid residue in 50 mL of 30% ethanol–water solution (v/v; ethyl acetate [EA] extract). The role of ethanol was to preserve extracts from microbial spoilage during the entire experimental period; however, both extracts were stored in a refrigerator.

OLE characterization

Total phenols (Folin–Ciocalteu method) and HPLC phenolic analysis were performed on both OLEs previously filtered through a 0.45-mm syringe and following the methods described by De Leonardis *et al.* (2022b) by using an independent hydroxytyrosol calibration curve (HyE: hydroxytyrosol equivalent) and by reading the absorbance at 760 nm with an Evolution TM201/220 instrument (Thermo Fischer Scientific SpA, Rodano, MI, Italy). The HPLC instrument used was a Varian ProStar 330 (Mulgrave, AUS) equipped with a Kinetex 5u C18 100 Å column (250 mm × 4.6 mm) (Phenomenex, USA). The mobile phase was a mixture of 0.2% H₃PO₄ (v/v) in bi-distilled water (eluent A), methanol (eluent B), and acetonitrile (eluent C), and the gradient for A/B/C eluents was as follows: 0 min, 96/2/2%; 24 min, 50/25/25%; 27 min, 40/30/30%; 36 min, 0/50/50%; and 49 min, 96/2/2%; chromatograms were obtained at 280 nm or 240 nm. The hydroxytyrosol and oleuropein peaks were identified by comparing their retention period and UV absorption characteristics with those of the corresponding commercial standards.

In vitro oxidation assays

For both DPPH and FRAP assays, the OLEs were initially diluted with water at concentrations ranging from 30 to 800 µg/mL. Comparable concentrations of pure

hydroxytyrosol were also prepared for comparison. DPPH free radical scavenging activity was determined by following the methods described by Williams *et al.* (1995) with slight modifications. A total of 7 µL methanol or Hy or OLE dilution was deposited in 300-µL wells of flat-bottomed polystyrene microplates, as a blank or samples, respectively; then, 273 µL of DPPH (60 µmol/L in methanol) was added to the microplate. Absorbance at 515 nm was measured using a plate reader (BioTek Elx 800, Agilent, Les Ulis, France) at intervals of 5 min. For the interpretation of results, when an absorbance plateau was reached, the percentage of residual DPPH° was calculated as a ratio between the value of the sample and the blank, and, finally, the concentration of antioxidant necessary to decrease the initial DPPH° concentration by 50% (EC₅₀) was calculated using the concentration–response regression.

For FRAP determination as described by Pulido *et al.* (2000), colorimetric reagent was freshly prepared by mixing the following reagents in a 10:1:1 (v/v) ratio: (i) acetate buffer (300 mM, pH 3.6); (ii) 2,4,6-tripyridyl-S-triazine (TPTZ) 10-mM solution in 40-mM HCl; and (iii) iron (III) chloride, 20 mM. For analysis, 300 µL of FRAP reagent, repositioned in microplate wells, was incubated at 37°C for 15 min, then 10 µL of each Hy or OLE dilution was added and mixed to FRAP reagent; absorbance at 593 nm was read after 4 min using a plate reader (BioTek Elx 800, Agilent, Les Ulis, France). A standard curve was established using an iron sulfate heptahydrate solution at concentrations ranging from 0 µM to 2,000 µM. FRAP values were expressed as mg Fe²⁺ equivalent per mL of extract. The EC₅₀ FRAP (half maximal effective concentration) was determined by developing a linear regression curve that connected the sample concentration (*x*-axis) with the FRAP value (*y*-axis).

Finally, the Rancimat test was performed with the Model 730 instrument (Metrohm AG, Herisau, Switzerland) using a lard sample purchased from the local market (Campobasso, Italy) and prepared without (control) and with OL extracts or Hy solution, all these added to give a 200-ppm dose of total phenol equivalent. The test conditions were set at 120°C and a constant air flow of 20 L/h by measuring induction time in hours.

Beef patties preparation and experimental design

For preparing patties, approximately 1 kg of under protective atmosphere-packaged minced beef meat was purchased from a local supermarket in Bourg-en-Bresse, France. The minced meat contained 20% fat, as declared on the label; it was further homogenized in the laboratory using a kitchen blender and then divided into three

equal parts. In part one, nothing was added (control sample); in part two, a calculated volume of WE or EA extract was added to achieve a concentration of 20 mg of total phenols for 100-g minced meat. Specifically, the added volume of WE or EA was, respectively, 3.0 mL or 1.9 mL for 100-g minced meat, corresponding to a final ethanol amount of 0.9% and 0.6%.

Subsequently, 15 g of every portion of minced meat was manually shaped into patties of identical form; all the patties were subsequently packaged in closed 1-L glass containers, in which the headspace composition differed as described below. In the set of samples, hereafter named 'natural air' (NA), the headspace was composed of uncontrolled atmospheric air; whereas, in the samples labeled 'high-oxygen-air' (HOA), the headspace was composed of 80% O₂ and 20% N₂, which was achieved by injecting O₂ into the container using the specific equipment Automate (AUTO Modified ATmosphere & Environment).

An adequate number of independent containers, suitable for replicated analysis, were prepared for each set of patty samples, different for the quantity of OLE added and/or the headspace composition.

In summary, the following acronyms were used for various sets of patty samples:

- CP-NA: control patties stored in natural-air headspace
- CP-HOA: control patties stored in high-oxygen-air headspace
- WEP-NA: patties with water extract stored in natural-air headspace
- WEP-HOA: patties with water extract stored in high-oxygen-air headspace
- EAP-NA: patties with ethyl acetate extract stored in natural-air headspace
- EAP-HOA: patties with ethyl acetate extract stored in high-oxygen-air headspace

Finally, all patty samples were stored in a refrigerator at 4°C; followed by analysis at intervals of 0, 5, and 10 days.

Measure of oxygen percentage in container headspace

The O₂ level percentage in the headspace was determined using a digital check pointer analyser. Fluorescent oxygen (SP-PSt3-NAU) pre-calibrated sensor spots (Presens GmbH, Regensburg, Germany) were attached to the inner surface of 1-L glass containers; O₂ concentration in the inner atmosphere of containers could thus be measured non-invasively with a Fibox trace (O₂) coupled with optical fiber reader along storage.

Physicochemical analysis of patties

pH was determined by inserting a pH meter electrode into filtered solution obtained by homogenization of 1 g patties with 10-mL deionized water.

Color of patty surface was evaluated using a colorimeter (CM-2300d, Konica Minolta, France) by assessing L* (lightness), a* (redness), and b* (yellowness) values and calibrating the instrument with zero and white standard plates before analysis.

Thiobarbituric acid-reactive substance (TBARS) assay

The oxidative stability of beef patties was determined by TBARS assay according to Nerin *et al.* (2006) with the following modification. Approximately, 2-g meat sample was weighed in a 50-mL falcon tube placed in crushed ice. Thus, 8 mL of 10% trichloroacetic acid, for protein precipitation, and 50 µL of BHT solution (1 mg/mL), for oxidation prevention, were added to the tube. The sample was homogenized using an Ultraturrax Grinder-Homogenizer (Ultra-Turrax, Janke & Kunkel, IKA-WERK, Germany) for at least 1 min at 20,000 rpm and then centrifuged (Sigma SIGMA 318KS, Sigma Laborzentrifugen GmbH, Germany) at 1,500 g for 30 min at 5°C; the recovered supernatant was filtered through a filter paper. For TBARS assay, 2 mL of filtrate was vortexed with 2 mL of TBA 2-thiobarbituric acid (20 mM) in Pyrex glass test tubes, which were closed with aluminium foil, incubated in a water bath at 95°C for 20 min, and then cooled immediately under a stream of cold water. Absorbance at 531 nm of the final solution was measured using a daily standard curve of malondialdehyde tetrabutylammonium (MDA) to calculate TBARS value expressed as mg of MDA per kg of patties.

Statistical analysis

All calculations were carried out with three independent measures for each set of samples by calculating means ± standard deviations with the Statistics 8.1 software. Two-way ANOVA and Tukey's honestly significant difference (HSD) test were performed to determine statistically significant differences between mean values ($p < 0.05$) using the same software.

Results and Discussions

Antioxidant capacity and characterization of olive leaf extracts

It is well known that phenols are secondary metabolites ubiquitous in the entire plant kingdom, including various

compounds, such as phenolic acids, flavonoids, stilbenes, and lignans, as well as in secoiridoids found in olive plants (McDonald *et al.*, 2001). Olive leaves have gained widespread use in various sectors, including medicine, cosmetics, and food products, because of their recognized functional properties derived from the presence of bioactive phenolic compounds. Different extraction techniques are applied to recover phenolic compounds from OL, such as liquid–liquid and solid-phase extraction, ultrasound and microwave-assisted extraction, and extraction with superheated solvent (Munekata *et al.*, 2020). Generally, synthetic solvents, such as ethers, chlorinated, and aromatic solvents are employed, raising concerns about their health and environmental impacts (Dobrinčić *et al.*, 2020).

In this study, a laboratory distiller was used with the initial aim of recovering an essential oil from OL. Only traces of essential oil were distilled whereas the water remaining in the upper part of the boiler appeared as red-brownish in color, indicating enrichment in phenolic compounds. Thus, we investigated the composition and potential application of phenolic compounds extracted by water steam, because it is a green solvent procedure.

Two different liquid OL extracts were prepared, hereafter abbreviated as WE and EA. WE was composed of a water-soluble bioactive compound that was dried and concentrated in a known volume of 30% ethanol–water solution (v/v). The EA extract represented the WE partially purified due to it contained only substances soluble in ethyl acetate; also in this case, the dry residue was re-dissolved in a known volume of 30% ethanol–water.

The phenol content and antioxidant capacity of OLEs are shown in Table 1, and the antioxidant capacity of pure hydroxytyrosol (Hy), assayed under the same conditions, is also shown for comparison.

Amount of total phenols was 7.4 HyE mg/mL and 3.8 HyE mg/mL in WE and EA, respectively. Boiling could have different effects on various classes of phenolic

compounds, but overall, it did not necessarily brought down total phenols extracted from OLs. These values are comparable with the findings of other studies when different extraction methods were performed (Boli *et al.*, 2022; De Leonardis *et al.*, 2015).

The DPPH radical scavenging assay is commonly used in antioxidant studies because of its simplicity and consistent results. It measures the ability of a sample to scavenge free radicals that cause oxidative stress. The assay involves mixing a stable DPPH radical with an antioxidant solution that donates electrons to the radical, causing a change in purple color intensity. The greater the number of donated electrons, the greater the antioxidant activity.

The data in Table 1 show the significant antioxidant potential of both OLEs. Specifically, the EC₅₀ values were 57.6 µg/mL and 76.5 µg/mL for WE and EA, respectively, while pure Hy was 39.7 µg/mL. A lower EC₅₀ value indicates higher radical scavenger activity, suggesting the superior efficacy of WE extract in combating free radicals and oxidative stress in comparison to EA extract. This is also visible when observing DPPH disappearance kinetics (data not shown): all tested concentrations were permitted to reach a plateau within 80 min for WE, while pure Hy and EA extracts needed up to 180 min for the lowest concentrations. Thus, the performance of WE suggested some synergistic effects. Moreover, for all tested substances, 20% of initial DPPH persisted, whatever was the concentration used, and all three behaviors were considered as slow kinetics. These results are comparable to those obtained with gallic acid (a commonly encountered phenolic acid in natural antioxidant plant extracts) under the same conditions (Williams *et al.*, 1995), thus establishing these extracts interesting and potent candidates for meat preservation.

Table 1 also reports the results relative to the FRAP assay, based on a single-electron transfer that causes the reduction of ferric iron (Fe³⁺) to ferrous iron (Fe²⁺). Effective concentration revealed that pure Hy standard exhibits

Table 1. Phenol content and antioxidant capacity of the olive leaf extracts (OLEs) and pure hydroxytyrosol (Hy).

Determinations	Water extract (WE)	Ethyl acetate extract (EA)	Hydroxytyrosol (Hy)	Lard*
Total phenols (TP) (mg/mL HyE)	7.4 ± 0.8	3.8 ± 0.3	1.0 ± 0.0	–
DPPH EC ₅₀ (µg/mL)	57.6 ± 0.4 ^a	76.5 ± 3.5 ^b	39.7 ± 0.8 ^c	–
FRAP EC ₅₀ (µg/mL)	84.8 ± 2.5 ^a	69.9 ± 4.1 ^b	41.2 ± 1.1 ^c	–
Induction time Rancimat test at 120°C (h)	8.92 ± 1.01 ^a	12.74 ± 2.03 ^b	7.27 ± 0.68 ^a	2.24 ± 0.19 ^c

Notes. Mean and standard deviations of three independent replicates for each set of samples. Different letters show significant difference on the same row ($p < 0.05$).

*Lard is the control sample without antioxidants.

the highest activity ($EC_{50} = 41.2 \mu\text{g/mL}$) followed by EA ($EC_{50} = 69.9 \mu\text{g/mL}$) and WE ($EC_{50} = 84.8 \mu\text{g/mL}$), suggesting that the diversity of molecules contained in the extracts resulted in different mechanisms of action against oxidation.

Finally, the antioxidant potential of OL extracts was evaluated directly in food fats using the Rancimat test at 120°C (Table 1). Both OLEs and pure Hy were added at a dose of 200 ppm HyE to a commercial lard sample. Surprisingly, both OLEs showed higher antioxidant capacities than that of Hy. Specifically, EA delayed oxidative stability of the lard by 5.7 times, followed by WE (4.0 times) and Hy (3.2 times). The Rancimat test results evidenced the good antioxidant potential of OLEs at the high temperatures reached while preparing meat patties.

By summarizing, at the same dose added of total phenol equivalent, both OLEs evidenced antioxidant activity *in vitro*, although their effectiveness varied with the oxidation test used. Specifically, WE exhibited higher radical scavenger activity than EA, which instead better performed using FRAP and Rancimat test.

Certainly, different performances of OLEs were correlated to their different phenolic profiles because of different polarity of the solvents (water and ethyl acetate) used for extraction process. Indeed, according to De Leonardis *et al.* (2015), ethyl acetate did not solubilize oxidized phenols, which are not usually detectable by

HPLC analysis, while the same reacts with the Folin–Ciocalteu reagent. In addition, these differences were also evident by comparing the color of OLE solutions, typically red for WE and yellow for EA (not shown).

In WE chromatogram (Figure 1), a high peak was well eluted in Hy-zone at approximately 10 min. We barred that this peak matched a Hy isomer because it showed a different spectrum; specifically, it was characterized by a maximum absorption at 240 nm, and any absorption at 280 nm typical of Hy and its isomers. Nevertheless, the above-mentioned peak was strongly resized in the EA chromatogram, in which numerous peaks eluted in the OLE zone. Identification and origin of this peak deserve future investigations; however, according to Cecchi *et al.* (2023), it is reasonable to suppose that it could correspond to an oleoside-like compound derived from oleuropein decomposition during the extraction process.

Oxygen percentage in container headspace

The storage test was carried out in natural (NA) and modified (HOA) atmospheres. In the NA set of patty samples, the headspace was composed of atmospheric air, which naturally contains about 78.0% N_2 , 20.9% O_2 , 0.9% Ar, and 0.1% CO_2 . Instead, in the HOA set of samples, with an aim to accelerate oxidative process for the excess of O_2 , the atmospheric air was modified artificially so to obtain a headspace composed of 80% O_2 and 20% N_2 .

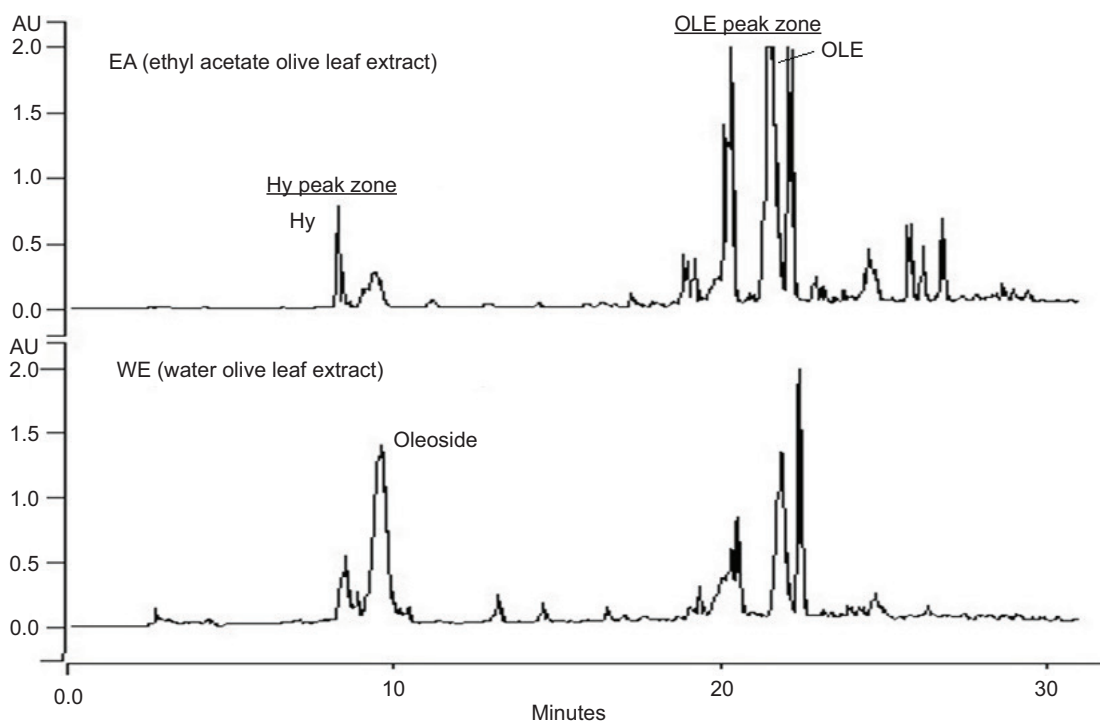


Figure 1. HPLC chromatogram of ethyl acetate (EA) and water (WE) olive leaf extracts determined at 240 nm.

Subsequent decrease in O₂ levels in headspace could be correlated with the activity of aerobic pathogens or spoilage microorganisms, or could also be an indication of leakage (Chibane *et al.*, 2017; Martin *et al.*, 2023). The observed variation of O₂ level in the storage bottle is shown in Figure 2.

Variation in O₂ percentage level was more evident in the set of samples with modified air. Moreover, a significant decrease in O₂ percentage was observed for the control set of patties because of leakage or respiration of meat flora. Specifically, O₂ percentage was almost halved at the end of the storage period (10 days), decreasing up to 10.5% and 48.3% in CP-NA and CP-HOA, respectively. Conversely, insignificant changes in O₂ percentage were observed in the containers with patties supplemented with WE and EA in both NA and HOA headspace (Figure 2).

In this study, the antimicrobial activity of the extracts was not evaluated; however, the maintaining of O₂ levels in the containers with WEP and EAP samples suggested a possible antimicrobial effect of OLEs in both high-O₂ and low-O₂ storage environments. In literature, a lot of studies highlighted the antimicrobial activity of OL phenolic compounds against major food-borne pathogens,

including *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Esherichia coli* O157:H7 (Liu *et al.*, 2017).

As regards the potential antimicrobial effect of OLE solvent, namely 30% ethanol–water solvent, it is reasonable to suppose that the real quantity of ethanol added to patties was insufficient to produce a significant antimicrobial effect. Indeed, the antimicrobial effect of pure ethanol in meat was less investigated, compared to other foods, such as bakery products, bread, and fruit drinks (Dao and Dantigny, 2011). A dose of at least 5% ethanol was found to be necessary to inhibit the growth of *Listeria monocytogenes* (Oh and Marshall, 1993) and molds (North *et al.*, 2019). Nevertheless, ethanol levels in WEP and EAP were estimated to be 0.9% and 0.6%, respectively, therefore, most likely, insufficient to have a possible antimicrobial effect in meat patty samples.

Physicochemical analysis of patties

pH changes

Data reported in Table 2 evidenced a trendy increase in pH during storage in the HOA set of samples, especially in CP-HOA, in which pH changed from a minimum of 5.3 at zero time to a maximum of 6.5 after 10 days.

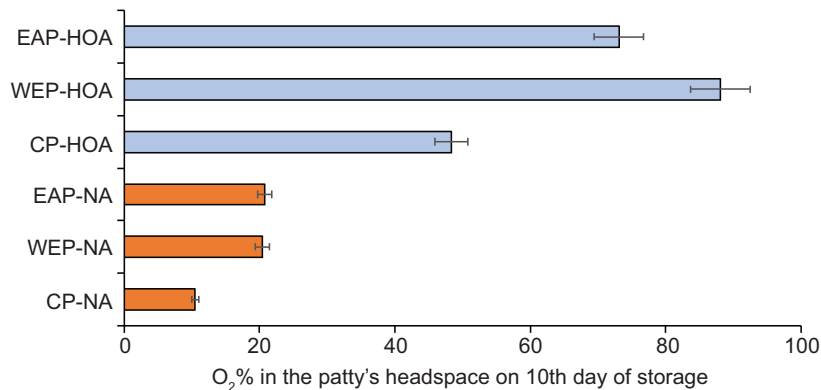


Figure 2. Oxygen percentage level measured in headspace of beef meat at the end of storage period (10 days).

Table 2. pH analysis of beef meat patties during storage at 4°C.

Storage days	Natural Air (NA)			High oxygen environment (HOA)		
	CP-NA	EAP-NA	WEP-NA	CP-HOA	WEP-HOA	EAP-HOA
0	5.3 ± 0.2 ^a	5.3 ± 0.2 ^a	5.3 ± 0.2 ^a	5.3 ± 0.1 ^a	5.2 ± 0.1 ^a	5.3 ± 0.1 ^a
5	5.2 ± 0.1 ^a	5.2 ± 0.1 ^a	5.2 ± 0.1 ^a	6.3 ± 0.1 ^b	6.0 ± 0.1 ^{b, c}	5.9 ± 0.1 ^c
10	5.5 ± 0.2 ^a	5.5 ± 0.2 ^a	5.5 ± 0.2 ^a	6.5 ± 0.3 ^b	5.9 ± 0.3 ^{c, d}	5.3 ± 0.3 ^d

CP-NA: control patties stored in natural-air headspace; EAP-NA: patties with ethyl acetate extract stored in natural-air headspace; WEP-NA: patties with water extract stored in natural-air headspace; CP-HOA: control patties stored in high-oxygen-air headspace; WEP-HOA: patties with water extract stored in high-oxygen-air headspace; EAP-HOA: patties with ethyl acetate extract stored in a high-oxygen air headspace. Mean and standard deviation values of three independent replicates for each set of samples. Different letters show significant difference on the same row ($p < 0.05$).

Conversely, no significant differences emerged in the set patties stored in natural air headspace containers (NA series), except for a slight pH increase on the 10th day.

North *et al.* (2019) considered that the increase in pH was likely due to the accumulation of alkaline breakdown products, such as amines and ammonia, caused by rabbit meat degradation during chilled storage. However, in the HOA set of samples, the positive effect of both OL extracts was evident in contrast to an increase in pH. Moreover, at the same dose added, the partially purified EA extracts showed a trendy higher performance than WE. According to Saleh *et al.* (2020), low pH of stored minced meat treated with OLEs could be a positive consequence of the nature and properties of the phenolic compound content.

Color analysis

Color is a sign of meat's quality and freshness and is one of the most significant sensory attributes that affects consumer acceptance. The color values determined for beef patties during storage are shown in Table 3.

According to Andres *et al.* (2016), the L* value, which measures the lightness of meat surface, depends on the combination of fat, pigment, and water content. Moreover, the L* value generally decreases during meat storage because of oxidation. A decrease in L* value was observed in this study, varying significantly with the headspace composition of container. In the set of patties stored in natural atmosphere (Table 3), the L* values of CP samples were lower than those found for the patties

containing OL extracts. Therefore, the antioxidant properties of OL extracts helped to maintain the lightness of patty surface during storage. These results were different from those of the samples stored in high-O₂ atmosphere (Table 3), in which a higher loss of L* values was observed in CP-HOA, WP-HOA, and EA-HOA. Therefore, in this case, OL extracts were not able to preserve the lightness of patties at an added dose.

The redness of fresh meat, measured as the a* value, is strongly affected by the content of myoglobin protein and O₂ exposure. Specifically, O₂ bonded to myoglobin stabilizes its red oxymyoglobin form whereas the oxidation of oxymyoglobin to brown metmyoglobin results in meat discoloration (Saleh *et al.*, 2020). One method for controlling food quality and safety is the application of new packaging systems, including modified atmosphere packaging (MAP). Kandeepan and Tahseen (2020) reported that high O₂ MAP levels (usually >70%) helped to maintain the bright red color of fresh meat and enhanced the marketability of minced meat. However, after prolonged storage, loss of redness may occur due to the conversion of oxymyoglobin to metmyoglobin (Hayes *et al.*, 2009). Consistent results were obtained in HOA set patties, for which an increase in redness a* values was found till the fifth day and then it decreased until the 10th day (Table 3). Redness is the most important parameter of red meat, as bright red color is highly appreciated by consumers. In a study conducted by Chibane *et al.* (2017), plant extracts from OLs, combined with high-oxygen-modified atmosphere packaging, maintained the redness index of meat, suggesting that this extract could delay color variation.

Table 3. Color characteristics of beef patties formulated with olive leaf extracts (OLEs) stored at 4°C under different headspace oxygen environment.

Storage days	Natural air (NA)			High oxygen environment (HOA)		
	CP-NA	EAP-NA	CP-NA	EAP-NA	CP-NA	EAP-NA
L*						
0	46.2 ± 0.7 ^a	47.4 ± 0.9 ^a	47.7 ± 0.8 ^a	44.2 ± 1.6 ^a	47.4 ± 1.2 ^a	49.5 ± 1.2 ^a
5	41.3 ± 0.7 ^b	54.9 ± 1.6 ^b	50.7 ± 1.5 ^b	38.2 ± 1.4 ^b	38.5 ± 1.3 ^b	45.6 ± 0.9 ^b
10	39.5 ± 0.5 ^b	43.5 ± 2.4 ^c	45.6 ± 0.4 ^c	34.6 ± 1.4 ^c	35.2 ± 1.2 ^c	35.5 ± 1.3 ^c
a*						
0	13.9 ± 0.5 ^a	13.2 ± 0.6 ^a	13.9 ± 0.5 ^a	14.0 ± 0.4 ^a	14.8 ± 0.4 ^a	13.4 ± 0.4 ^a
5	9.9 ± 0.7 ^b	11.3 ± 0.5 ^b	10.6 ± 0.7 ^b	16.3 ± 1.8 ^b	15.3 ± 1.1 ^a	13.9 ± 1.0 ^b
10	8.3 ± 0.3 ^b	9.1 ± 1.9 ^c	8.9 ± 0.7 ^c	6.7 ± 0.9 ^c	8.8 ± 1.3 ^c	7.2 ± 1.6 ^c
b*						
0	11.9 ± 1.6 ^a	10.5 ± 2.4 ^a	9.1 ± 0.7 ^a	13.6 ± 0.3 ^a	12.6 ± 0.6 ^a	12.7 ± 0.4 ^a
5	13.6 ± 0.4 ^b	11.2 ± 1.0 ^{a,b}	11.3 ± 0.9 ^{a,b}	12.4 ± 0.6 ^b	11.7 ± 0.4 ^{a,b}	10.7 ± 1.4 ^b
10	16.0 ± 0.4 ^c	13.9 ± 2.3 ^b	10.5 ± 4.9 ^b	14.0 ± 0.3 ^{a,b}	15.6 ± 2.6 ^c	12.8 ± 2.0 ^a

CP-NA: control patties stored in natural-air headspace; EAP-NA: patties with ethyl acetate extract stored in natural-air headspace. Different letters show significant difference on the same column for L*, a*, and b* (p < 0.05).

The yellowness (b^* values) of beef patties increased with time, and both treatment and storage significantly affected the b^* value ($p > 0.05$). Higher b^* value was observed for control patties, which could be due to protein oxidation, leading to the formation of yellowish compounds, altering the appearance of meat. A similar result was found in a study conducted by Xia *et al.* (2021), which assumed that myoglobin, the protein responsible for the red color of meat, could oxidise over time, leading to a brown color. This oxidation process indirectly affects the b^* value, because the redness (a^* value) decreases, making the yellowness more pronounced. In general, the color of treated beef patties could be affected not only by the presence of natural pigments in extracts but also by the ability of phenolic compounds to maintain myoglobin in a reduced state.

Lipid oxidation

Oxidation of polyunsaturated fatty acids is the principal cause of lipid deterioration. An oxidative process involves air O_2 that promotes the formation of radical oxygen species (ROS). The reaction proceeds through multiple stages, such as initiation, propagation, and termination, and produces various primary and secondary molecules, also potentially toxic, that have implications for meat quality as well as human health.

TBARS is a common marker for studying the progress of lipid oxidation also used as an indicator of freshness in meat (Allam *et al.*, 2024; Xia *et al.*, 2021).

TBARS values determined in patties during storage are given in Figure 3.

Regarding the CP samples prepared without any additives, significant increase in TBARS was observed depending on both storage period and headspace conditions. Specifically, TBARS measures of CP-NA showed an increasing trend of 0.52 mg MDA/kg, 0.70 mg MDA/kg, and 0.78 mg MDA/kg at 0, 5, and 10 days of storage, respectively. A similar trend, but accelerated, was observed in the CP-HOA set of samples, in which TBARS values increased from 0.55 mg MDA/kg (0 day) to 1.31 mg MDA/kg (10 days). Thus, as expected, the O_2 supplement in container accelerated significantly the TBARS formation in CP-HOA samples during storage.

From the beginning (0 day) and under both headspace conditions, TBARS values of the patties containing 200 ppm of total phenols derived from both WE and EA extracts were significantly lower than those of the corresponding CP.

The starting TBARS value for all patties with OLEs was about 0.22 mg MDA/kg on average, without exhibiting any significant difference irrespective of the type of OLE added. This value was about half of the value of the corresponding CP, evidencing that lipid oxidation occurred in control patties during preparation. Conversely, OLEs slowed down lipid autoxidation during the manipulation of minced meat to form patties. The antioxidant

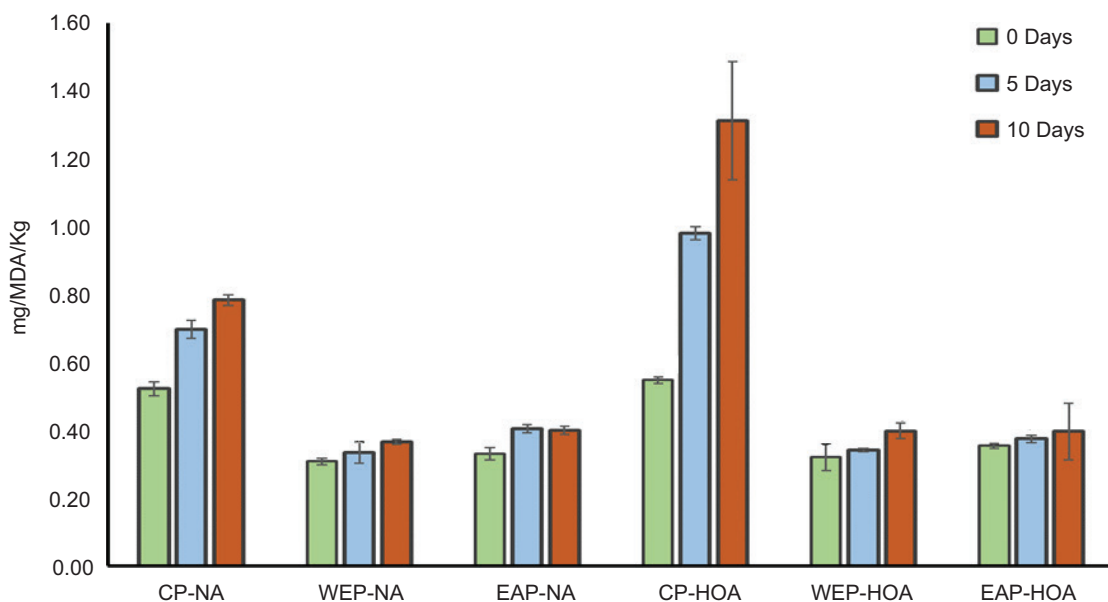


Figure 3. TBARS analysis. Changes in MDA determined in different beef patties during storage at 4°C. Mean and standard deviation values of three independent replicates for each set of samples. Different letters indicate significant differences at $p < 0.05$.

effect of OLEs continued during the storage of patties when TBARS values substantially remained unchanged for up to 10 days under both NA and HOA conditions (Figure 3).

Thus, the effectiveness of both OLEs in delaying lipid oxidation in patties was well highlighted. Phenolic compounds are certainly responsible for the antioxidant performance of OLEs as already observed in other studies (Kurt and Ceylan, 2017; Totaro *et al.*, 2024).

Conclusions

To summarize, the antioxidant capacity *in vitro* and of minced beef meat of the studied OLEs was demonstrated.

Specifically, the extracts were efficient in delaying lipid oxidation and enhancing physicochemical properties of minced beef patties, including pH and color, during refrigerated storage for up to 10 days. Thus, these extracts could be evaluated as possible alternative natural antioxidants to enhance the quality and shelf-life of meat.

Olive leaves, generally less used, are a copious by-product of the olive oil production chain. The proposed utilization of OLs represents a possible form of valorization of this by-product by following the circular economy principles. Moreover, OLEs could be produced in laboratory through a simple and green process based on the application of water steam. We proved that the applied extraction procedure did not affect the antioxidant potential of phenolic compounds occurring naturally in OLs. Certainly, the method followed could be improved as well as tested on other vegetable wastes rich in bioactive substances.

For the above-mentioned reasons, the studied OLEs meet the sustainability objectives. However, the promising results open options for further research to fully understand the benefits, particularly regarding the microbiological safety of meat, which is a critical aspect of food preservation as well as public health.

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Conflict of Interest

The authors declared no conflict of interest.

References

- Allam, A.Y., Abdi, G., Badr, A.A., Abbasi Tarighat, M. and Rafat Elkabary, M. 2024. antioxidant and antibacterial effect of lemon verbena leaves (*Lippia citriodora*) extract as a natural preservative on refrigerated meat patties during storage. Ital J Food Sci. 36(3): 95–114. <https://doi.org/10.15586/ijfs.v36i3.2514>
- Andres, A.I., Petron, M.J., Delgado-Adamez, J., Lopez, M. and Timon, M. 2016. Effect of tomato pomace extracts on the shelf-life of modified atmosphere-packaged lamb meat. J Food Process Preserv. 41(4): e13018. <https://doi.org/10.1111/jfpp.13018>
- Aouidi F., Okba A., Hamdi M. Valorization of functional properties of extract and powder of olive leaves in raw and cooked minced beef meat. J Sci Food Agric 2017;97: 3195–3203. <https://doi.org/10.1002/jsfa.8164>
- Bender, C., Strassmann, S. and Golz, C. 2023. Oral bioavailability and metabolism of hydroxytyrosol from food supplements. Nutrients. 15: 325. <https://doi.org/10.3390/nu15020325>
- Boli, E., Prinos, N., Louli, V., Pappa, G., Stamatis, H. and Magoulas, K. 2022. Recovery of bioactive extracts from olive leaves using conventional and microwave-assisted extraction with classical and deep eutectic solvents. Separations. 9(9): 255. <https://doi.org/10.3390/separations9090255>
- Carocho, M., Morales, P. and Ferreira, I.C.F.R. 2018. Antioxidants: reviewing the chemistry, food applications, legislation, and role as preservatives. Trends Food Sci Technol. 71: 107–120. <https://doi.org/10.1016/j.tifs.2017.11.008>
- Cecchi, L., Ghizzani, G., Bellumori, M., Lammi, C., Zanoni, B. et al. 2023. Virgin olive oil by-product valorization: an insight into the phenolic composition of olive seed extracts from three cultivars as sources of bioactive molecules. Molecules. 28(6): 2776. <https://doi.org/10.3390/molecules28062776>
- Chibane, B.L., Ouled-Bouhedda, B., Leonard, L., Gemelas, L., Bouajila, J., Ferhout, H. and Oulahal, N. 2017. Preservation of fresh ground beef patties using plant extracts combined with a modified atmosphere packaging. Eur Food Res Technol. 243: 1997–2009. <https://doi.org/10.1007/s00217-017-2905-3>
- Dao, T. and Dantigny, P. 2011. Control of food spoilage fungi by ethanol. Food Control. 22(3–4): 360–368. <https://doi.org/10.1016/j.foodcont.2010.09.019>
- De Leonardis, A., Iftikhar, A. and Macciola, V. 2022a. Possible utilization of two-phase olive pomace (TPOP) to formulate potential functional beverages: a preliminary study. Beverages. 8(3): 57. <https://doi.org/10.3390/beverages8030057>
- De Leonardis, A., Macciola, V., Cuomo, F. and Lopez, F. 2015. Evidence of oleuropein degradation by olive leaf protein extract. Food Chem. 175: 568–574. <https://doi.org/10.1016/j.foodchem.2014.12.016>
- De Leonardis, A., Macciola, V., Iftikhar, A. and Lopez, F. 2022b. Characterization, sensory and oxidative stability analysis of vegetable mayonnaise formulated with olive leaf vinegar as an active ingredient. Foods. 11(24): 4006. <https://doi.org/10.3390/foods11244006>
- De Leonardis, A., Macciola, V. and Nag, A. 2009. Antioxidant activity of various phenol extracts of olive-oil mill wastewaters. Acta Aliment. 38(1): 77–86. <https://doi.org/10.1556/aalim.2008.0030>

- Difonzo, G., Totaro, M.P., Caponio, F., Pasqualone, A. and Summo, C. 2022. Olive leaf extract (OLE) addition as tool to reduce nitrate and nitrite in ripened sausages. *Foods*. 11(3): 451. <https://doi.org/10.3390/foods11030451>
- Djenane, D., Gómez, D., Yangüela, J., Roncalés, P. and Ariño, A. 2018. Olive leaves extract from Algerian oleaster (*Olea europaea* var. *sylvestris*) on microbiological safety and shelf-life stability of raw halal minced beef during display. *Foods*. 8(1): 10. <https://doi.org/10.3390/foods8010010>
- Dobrinčić, A., Repajić, M., Garofulić, I.E., Tuđen, L., Dragović-Uzelac, V. and Levaj, B. 2020. Comparison of different extraction methods for the recovery of olive leaves polyphenols. *Processes*. 8(9): 1008. <https://doi.org/10.3390/pr8091008>
- Gómez, I., García-Varona, C., Fernández, C.M. and Heras, O.M. 2020. Effects of an extract from olive fruits on the physicochemical properties, lipid oxidation and volatile compounds of beef patties. *Foods*. 9(12): 1728. <https://doi.org/10.3390/foods9121728>
- Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Marino Gammazza, A., Knap, N.; Wozniak, M. and Gorska-Ponikowska, M. 2018. Potential health benefits of olive oil and plant polyphenols. *Int J Mol Sci*. 19(3): 686. <https://doi.org/10.3390/ijms19030686>
- Hadidi, M., Palacios, O.J.C., Aghababaei, F., Serrano, S.D.J., Moreno, A. and Lorenzo, J.M. 2022. Plant by-product antioxidants: control of protein-lipid oxidation in meat and meat products. *LWT Food Sci Technol*. 169: 114003. <https://doi.org/10.1016/j.lwt.2022.114003>
- Hayes, J.E., Stepanyan, V., Allen, P., O'Grady, M.N., O'Brien, N.M. and Kerry, J.P. 2009. The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. *Meat Sci*. 83(2): 201–208. <https://doi.org/10.1016/j.meatsci.2009.04.019>
- Honikel, K.O. 2017. Oxidative changes and their control in meat and meat products. In: Toldrà, F. (Ed.) *Safety of Meat and Processed Meat*. Springer, New York, NY; pp. 313–340. https://doi.org/10.1007/978-0-387-89026-5_12
- Kandeepan, G. and Tahseen, A. 2022. Modified atmosphere packaging (MAP) of meat and meat products: a review. *J Packag Technol Res*. 6(3): 137–148. <https://doi.org/10.1007/s41783-022-00139-2>
- Kurt, Ş. and Ceylan, H.G. 2017. Effects of olive leaf extract on the oxidation stability and microbiological quality of dry fermented sausage (Sucuk). *Carpathian J Food Sci Technol*. 9(4): 178–188. <https://doi.org/10.3136/fstr.16.191>
- Liu, Y., McKeever, L.C. and Malik, N.S. 2017. Assessment of the antimicrobial activity of olive leaf extract against food-borne bacterial pathogens. *Front Microbiol*. 8: 113. <https://doi.org/10.3389/fmicb.2017.00113>
- Lorenzo, J.M., Domínguez, R. and Carballo, J. 2017. Control of lipid oxidation in muscle food by active packaging technology. In: Banerjee, R., Verma, A.K. and Siddiqui, M.W. (Eds.) *Natural Antioxidants: Applications in Foods of Animal Origin*. Apple Academic Press, Boca Raton, FL; pp. 343–382. <https://doi.org/10.1201/9781315365916-10>
- Martin, D., Joly, C., Dupas-Farrugia, C., Adt, I., Oulahal, N. and Degraeve, P. 2023. Volatilome analysis and evolution in the headspace of packed refrigerated fish. *Foods*. 12(14): 2657. <https://doi.org/10.3390/foods12142657>
- McDonald, S., Prenzler, P.D., Antolovich, M. and Robards, K. 2001. phenolic content and antioxidant activity of olive extracts. *Food Chem*. 73: 73–84. [https://doi.org/10.1016/S0308-8146\(00\)00288-0](https://doi.org/10.1016/S0308-8146(00)00288-0)
- Munekata, P.E., Nieto, G., Pateiro, M. and Lorenzo, J. 2020. M. Phenolic compounds obtained from *Olea europaea* by-products and their use to improve the quality and shelf life of meat and meat products. A review. *Antioxidants*. 9(11): 1061. <https://doi.org/10.3390/antiox9111061>
- Nerín, C., Tovar, L., Djenane, D., Camo, J., Salafranca, J. et al. 2006. Stabilization of beef meat by a new active packaging containing natural antioxidants. *J Agric Food Chem*. 54(20): 7840–7846. <https://doi.org/10.1021/jf060775c>
- North, M.K., Dalle Zotte, A. and Hoffman, L.C. 2019. The effects of dietary quercetin supplementation on the meat quality and volatile profile of rabbit meat during chilled storage. *Meat Sci*. 158: 107905. <https://doi.org/10.1016/j.meatsci.2019.107905>
- Oh, D.H. and Marshall, D.L. 1993. Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against *Listeria monocytogenes*. *Int J Food Microbiol*. 20(4): 239–246. [https://doi.org/10.1016/0168-1605\(93\)90168-g](https://doi.org/10.1016/0168-1605(93)90168-g)
- Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentao, P. and Andrade, P.B. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrancosa) leaves. *Molecules*. 12: 1153–1162. <https://doi.org/10.3390/12051153>
- Pulido, R., Bravo, L. and Calixto, F.S. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem*. 48: 3396–3402. <https://doi.org/10.1021/jf9913458>
- Rahmanian, N., Jafari, S.M. and Wani, T.A. 2015. Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. *Trends Food Sci Technol*. 42: 150–172. <https://doi.org/10.1016/j.tifs.2014.12.009>
- Romani, A.; Ieri, F., Urciuoli, S., Noce, A., Marrone, G., Nediani, C. and Bernini, R. 2019. Health effects of phenolic compounds found in extra-virgin olive oil, by-products, and leaf of *Olea europaea* L. *Nutrients*. 11: 1776. <https://doi.org/10.3390/nu11081776>
- Ruzzolini, J., Peppicelli, S., Andreucci, E., Bianchini, F., Scardigli, A., Romani, A., La Marca, G., Nediani, C. and Calorini, L. 2018. Oleuropein, the main polyphenol of *Olea europaea* leaf extract, has an anti-cancer effect on human BRAF melanoma cells and potentiates the cytotoxicity of current chemotherapies. *Nutrients*. 10: 1950. <https://doi.org/10.3390/nu10121950>
- Saleh, E., Morshdy, A.E., El-Manakhly, E., Al-Rashed, S., Hetta, H.F., Jeandet, P., Yahia, R., El-Saber Batiha, G. and Ali, E. 2020. Effects of olive leaf extracts as natural preservative on retailed poultry meat quality. *Foods*. 9(8): 1017. <https://doi.org/10.3390/foods9081017>
- Shimizu, H. and Iwamoto, S. 2022. Problems of lipid oxidation in minced meat products for a ready-made meal during cooking, processing, and storage. *Rev Agri Sci* 10: 24–35. https://doi.org/10.7831/ras.10.0_24
- Totaro, M.P., Difonzo, G., Pasqualone, A. and Summo, C. 2024. Physicochemical properties and sensory features of ripened,

- industrially prepared sausages, enriched with olive leaf extract to replace nitrite and nitrate. *LWT Food Sci Technol (LWT)*. 196: 115852. <https://doi.org/10.1016/j.lwt.2024.115852>
- Tzamaloukas, O., Neofytou, M.C. and Simitzis, P.E. 2021. Application of olive by-products in livestock with emphasis on small ruminants: implications on rumen function, growth performance, milk and meat quality. *Animals*. 11(2): 531. <https://doi.org/10.3390/ani11020531>
- Williams, B.W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol (LWT)*. 28: 25–30. [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5)
- Xia, C., Wen, P., Yuan, Y., Yu, X., Chen, Y., Xu, H., Cui, G. and Wang, J. 2021. Effect of roasting temperature on lipid and protein oxidation and amino acid residue side chain modification of beef patties. *RSC Adv*. 11(35): 21629–21641. <https://doi.org/10.1039/d1ra03151a>
- Zhang, Y., Zhang, Y., Jia, J., Peng, H., Qian, Q., Pan, Z. and Liu, D. 2023. Nitrite and nitrate in meat processing: functions and alternatives. *Curr Res Food Sci*. 6: 100470. <https://doi.org/10.1016/j.crf.2023.100470>